

## HuR and post-transcriptional regulation in vascular aging

WANG WenGong

*Research Center on Aging, Department of Biochemistry and Molecular Biology, Peking University Health Science Center, Beijing 100191, China*

Received May 4, 2014; accepted June 16, 2014

HuR (ELAV11 (embryonic lethal, abnormal vision)-like 1), a ubiquitously expressed member of the ELAV-like RNA-binding protein family, has been shown to regulate the stability and translation of mRNAs that encode factors regulating cellular senescence, thereby impacting on aging. In this review, we discuss the current knowledge of HuR's role in vascular cell senescence and vascular aging.

**HuR, mRNA turnover, translation, cell senescence, vascular aging**

**Citation:** Wang WG. HuR and post-transcriptional regulation in vascular aging. *Sci China Life Sci*, 2014, 57: 863–866, doi: 10.1007/s11427-014-4706-2

Vascular cell senescence is defined as a state of indefinite growth cessation that accompanies the replicative exhaustion of cells or growth arrest triggered by cellular damage or oncogenic signaling [1]. It is accepted that vascular cell senescence contributes to vascular aging, a process of exhausted vascular function accompanied by changes including luminal dilation, increase in arterial stiffness, endothelial dysfunction, and diffuse intimal thickening [2]. The expression of senescence-associated genes that govern the progression of aging is regulated at multiple levels. In addition to transcriptional mechanisms, studies over the past decade have revealed that post-transcriptional gene regulation, especially through changes in mRNA turnover and translation, critically influences protein expression patterns in the senescent cell [1].

Although the mechanisms controlling mRNA turnover or translation are not fully understood, interactions between regulatory factors and mRNAs are thought to be important for post-transcriptional regulation. The regulatory factors involved in mRNA turnover and translation include regulatory RNA binding proteins, microRNAs and long non-

coding RNAs (lncRNAs) [1,3,4]. HuR, a ubiquitously expressed member of the Hu RNA-binding protein family, has been widely shown to stabilize various mRNAs. HuR also acts as an important regulator of translation and nuclear export of target mRNAs [1]. The molecular distinction between the function of HuR as a stabilizing factor for some target mRNAs and a factor that modulates translation of other target mRNAs is not understood in detail. It is well established that HuR binds to mRNAs bearing U-rich or AU-rich sequences (AREs), which are typically present in their 3'-untranslated regions (3'-UTRs) [5]. This review focuses on the RNA-binding protein HuR, which is involved in the regulation of turnover or translation of mRNAs encoding vascular aging-related proteins.

### 1 Post-transcriptional regulation in vascular cell senescence

The expression of senescence-associated genes is of critical importance for governing the progression towards and the maintenance of vascular senescence. Examples of genes whose expression is elevated during vascular aging or in

\*Corresponding author (email: wwg@bjmu.edu.cn)

senescent vascular cells include p16<sup>INK4</sup>, p21<sup>CIP1</sup>, p27<sup>KIP1</sup>, ICAM-1, TGF-β2, GADD153, PAI-1, IGFBP-5 and fibronectin, while genes whose expression is reduced include SIRT1, eNOS, cyclin A, cyclin B1, cdc2, Id-1, and DHP1 [6–8]. The regulation of aging-related genes at the transcriptional level has been intensively studied for several decades. Additionally, studies over the past decade have revealed that post-transcriptional regulation, especially through control of mRNA turnover and translation, critically influences protein expression patterns in the senescent cell [1,9]. Among the post-transcriptional regulatory factors, the RNA-binding protein HuR is particularly influential in the establishment of senescence-associated protein profiles.

**2    HuR and post-transcriptional regulation in vascular cell senescence**

Proliferation arrest is a common and typical characteristic of cell senescence [10]. Therefore, genes involved in the regulation of cell division are regulators of cell senescence. Interestingly, HuR was described as a regulator of the cell cycle, at least in part, by mediating cell cycle-dependent stabilization of mRNAs encoding cyclins A and B1 (Table 1) [11]. During cell senescence, the loss of HuR function reduces the stability of mRNAs encoding cyclin A, cyclin B1, and c-fos, which in turn causes a reduction in the expression of these cell cycle regulators and enhanced cellular senescence [12]. In different cell types, elevation of the CDK inhibitor p16 contributes to proliferation arrest and the senescence phenotype. HuR was found to bind to the p16<sup>INK4</sup> 3'-UTR and to recruit AUF1 and Ago2 (an important component of the RNA-induced silencing complex (RISC)). This led to the destabilization of the p16 mRNA in replicative senescence (Table 1) [13]. The secondary structure of the p16 3'-UTR is important for the function of HuR, be-

cause disruption of the secondary structure by point mutation completely abolished the effect of HuR in destabilizing p16 mRNA [13]. In a recent study, the tRNA methyltransferase NSun2 was shown to methylate the p16 3'-UTR at A988 (m6A). Methylation by NSun2 antagonizes the interaction of HuR with the p16 3'-UTR, thereby stabilizing the p16 3'-UTR. In oxidative stress-induced cell senescence, the NSun2-p16 regulatory process is responsible for elevating p16 levels [14]. p27<sup>KIP1</sup> is another important gene for the growth and senescence of vascular endothelial and vascular smooth muscle cells [15,16]. HuR was also described as a repressor of p27 of translation (Table 1). The repression of p27 translation by HuR may depend on the association of HuR with the internal ribosomal entry site (IRES) located in the p27 5'-UTR [17]. The impact of HuR upon the above regulatory factors of the cell division cycle has not been reported with respect to vascular aging; however, this regulation may be important for this process because reduced levels of HuR during vascular aging are accompanied by decreased levels of cyclin A and cyclin B1 as well as increased levels of p16 and p27 [6,7,18].

Apart from the aforementioned target mRNAs, HuR has also been reported to regulate the expression of SIRT1, TNF-α, IL-6, IL-8, eNOS, ICAM1, VCAM-1, and VEGF (Table 1) [9–23]. HuR can stabilize the mRNAs of SIRT1, TNF-α, IL-6, IL-8, and VEGF by recognizing the AREs located in the 3'-UTRs of these mRNAs [19,20,22,23]. Reduced levels of SIRT1 were observed in senescent human diploid fibroblasts, and SIRT1 has a well-established impact upon senescence, certain diseases and life-span [24,25]. Reduction of VEGF levels has also been described as an important event in vascular aging, and VEGF can prevent human vascular endothelial cells from senescence [8]. Because HuR, SIRT1 and VEGF levels decline with vascular aging [6,8,24], it is possible that the reduced SIRT1 and VEGF levels in senescent cells may result from the loss of

**Table 1**    Role of HuR in the regulation of vascular aging-related genes

Target genes	mRNA stability		Translation efficiency	
	Increase	Decrease	Increase	Decrease
cyclin A	Increase [10,11]			
cyclin B1	Increase [10,11]			
p16		Decrease [12]		
p27				Decrease [14,15]
TNF-alpha	Increase [18]			
VEGF	Increase [19]			
eNOS	ND	ND	ND	ND [20]
SIRT1	Increase [21]			
IL-8	Increase [25]			
IL-6	Increase [25]			
ICAM-1	Increase [27]			
VCAM-1	Increase [27]			
COX2	Increase [29]			

a) The vascular aging-related genes whose expressing is regulated by HuR are listed. The regulation level (mRNA stability or translation), effect (increase or decrease), and the references are presented. ND, not determined.

HuR-mediated mRNA stabilization. Endothelial nitric oxide synthase (eNOS), ICAM-1 and VCAM-1 are also targets of HuR. Indeed, a study by Rhee et al. [21] indicated that HuR may regulate eNOS, ICAM-1, and VCAM-1 by activating NF- $\kappa$ B. There is no supporting evidence for the direct association of HuR to eNOS mRNA; however, HuR is unable to influence the translocation of NF- $\kappa$ B directly; therefore, HuR may directly bind to eNOS mRNA and regulate its turnover or translation. This, however, requires confirmation. In summary, by regulating vascular senescence-associated genes (Table 1), HuR may act as a regulator of vascular cell senescence.

### 3 HuR and *in vivo* vascular aging

By secreting numerous cytokines, growth factors, and proteases (the senescence-associated secretory phenotype (SASP)), senescent cells contribute to tissue repair, tumor promotion, and *in vivo* aging [26]. Given that HuR regulates genes controlling vascular cell aging, studies have been conducted to investigate whether HuR influences the process of *in vivo* vascular aging. In aged rats, the expression of HuR in the aorta was significantly decreased at the mRNA and protein levels [8]. Treatment of apolipoprotein-E-deficient mice with TNF- $\alpha$  induced HuR translocation and interaction between HuR and the 3'-UTR of ICAM-1 mRNA, thereby increasing ICAM-1 expression and enhancing leukocyte adhesion [27]. Phosphorylation of HuR by PKC enhances HuR binding to target mRNAs [28,29]. In the retina of streptozotocin (STZ)-induced diabetic rats, PKC $\beta$ /HuR activation was accompanied by enhanced VEGF protein expression that was blunted by a PKC $\beta$  inhibitor [30]. Therefore, HuR may be an important player in the process of *in vivo* vascular aging.

### 4 Conclusion

The altered abundance of genes expressed during vascular cell senescence determines the progression of cell aging. Undoubtedly, future studies will identify additional genes involved in senescence and HuR may target more mRNAs than we are currently aware of. Although the mechanisms underlying HuR-mediated regulatory events remain largely unknown, it is accepted that HuR often influences the fate of target mRNAs by working in association with other RNA-binding proteins and post-transcriptional factors (e.g., microRNAs) [13,31,32]. The association of HuR with other post-transcriptional factors and the influence of these associations on the regulation of genes involved in vascular aging should be carefully studied. In addition, regulation of HuR translation by miR-519, miR-146, miR-16, miR-125a and HuR itself have been reported [33–38]. The miR-519-HuR and HuR-HuR regulatory processes impact

on the expression of HuR in the replicative senescence of human diploid fibroblast [33,35]. The impact of the above regulatory processes in vascular aging or age-related decline therefore needs to be further investigated.

*This work was supported by the National Natural Science Foundation of China (81230008, 91339114) and 111 project of Ministry of Education of China (B07001).*

- 1 Wang W. Regulatory RNA-binding proteins in senescence. *Ageing Resh Rev*, 2012, 11: 485–490
- 2 Laurent S. Defining vascular aging and cardiovascular risk. *J Hypertens*, 2012, 30: S3–8
- 3 Inukai S, Slack F. microRNAs and the genetic network in aging. *J Mol Biol*, 2013, 425: 3601–3608
- 4 Yoon JH, Abdelmohsen K, Gorospe M. Posttranscriptional gene regulation by long noncoding RNA. *J Mol Biol*, 2013, 425: 3723–3730
- 5 Peng SS, Chen CY, Xu N, Shyu AB. RNA stabilization by the AU-rich element binding protein, HuR, an ELAV protein. *EMBO J*, 1998, 17: 3461–3470
- 6 Minamino T, Miyauchi H, Yoshida T, Tateno K, Kunieda T, Komuro I. Vascular cell senescence and vascular aging. *J Mol Cell Cardiol*, 2004, 36: 175–183
- 7 Foreman KE, Tang J. Molecular mechanisms of replicative senescence in endothelial cells. *Exp Gerontol*, 2003, 38: 1251–1257
- 8 Watanabe Y, Lee SW, Detmar M, Ajioka I, Dvorak HF. Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) delays and induces escape from senescence in human dermal microvascular endothelial cells. *Oncogene*, 1997, 14: 2025–2032
- 9 Brewer G. Messenger RNA decay during aging and development. *Ageing Res Rev*, 2002, 1: 607–625
- 10 Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res*, 1961, 25: 585–621
- 11 Wang W, Caldwell MC, Lin S, Furneaux H, Gorospe M. HuR regulates cyclin A and cyclin B1 mRNA stability during cell proliferation. *EMBO J*, 2000, 19: 2340–2350
- 12 Wang W, Yang X, Cristofalo V, Holbrook N, and Gorospe M. Loss of HuR is linked to reduced expression of proliferative genes during replicative senescence. *Mol Cell Biol*, 2001, 21: 5889–5898
- 13 Chang N, Yi J, Guo G, Liu X, Shang Y, Tong T, Cui Q, Zhan M, Gorospe M, Wang W. HuR uses AUF1 as a cofactor to promote p16<sup>INK4</sup> mRNA decay. *Mol Cell Biol*, 2010, 30: 3875–3886
- 14 Zhang X, Liu Z, Yi J, Tang H, Xing J, Yu M, Tong T, Shang Y, Gorospe M, Wang W. NSun2 stabilizes p16<sup>INK4</sup> mRNA by methylating the p16 3'UTR. *Nat Commun*, 2012, 3: 712
- 15 Werth D, Grassi G, Konjer N, Dapas B, Farra R, Giansante C, Kandolf R, Guarnieri G, Nordheim A, Heidenreich O. Proliferation of human primary vascular smooth muscle cells depends on serum response factor. *Eur J Cell Biol*, 2010, 89: 216–224
- 16 Li M, Chiu JF, Gagne J, Fukagawa NK. Age-related differences in insulin-like growth factor-I receptor signaling regulates Akt/FOXO3a and ERK/Fos pathways in vascular smooth muscle cells. *J Cell Physiol*, 2008, 217: 377–387
- 17 Kullmann M, Göpfert U, Siewe B, Hengst L. ELAV/Hu proteins inhibit p27 translation via an IRES element in the p27 5'UTR. *Genes Dev*, 2012, 16: 3087–3099
- 18 Klöss S, Rodenbach D, Borden R, Mülsch A. Human-antigen R (HuR) expression in hypertension: downregulation of the mRNA stabilizing protein HuR in genetic hypertension. *Hypertension*, 2005, 45: 1200–1206
- 19 Dean JL, Wait R, Mahtani KR, Sully G, Clark AR, Saklatvala J. The 3' untranslated region of tumor necrosis factor alpha mRNA is a target of the mRNA-stabilizing factor HuR. *Mol Cell Biol*, 2001, 21: 721–730
- 20 Levy NS, Chung S, Furneaux H, Levy AP. Hypoxic stabilization of

- vascular endothelial growth factor mRNA by the RNA-binding protein HuR. *J Biol Chem*, 1998, 273: 6417–6423
- 21 Rhee WJ, Ni CW, Zheng Z, Chang K, Jo H, Bao G. HuR regulates the expression of stress-sensitive genes and mediates inflammatory response in human umbilical vein endothelial cells. *Proc Natl Acad Sci USA*, 2010, 107: 6858–6863
  - 22 Abdelmohsen K, Pullmann R Jr., Lal A, Kim HH, Galban S, Yang X, Blethrow JD, Walker M, Shubert J, Gillespie DA, Furneaux H, Gorospe M. Phosphorylation of HuR by Chk2 regulates SIRT1 expression. *Mol Cell*, 2007, 25: 543–557
  - 23 Nabors LB, Gillespie GY, Harkins L, King PH. HuR, a RNA stability factor, is expressed in malignant brain tumors and binds to adenine- and uridine-rich elements within the 3' untranslated regions of cytokine and angiogenic factor mRNAs. *Cancer Res*, 2001, 61: 2154–2161
  - 24 Chen HZ, Wan YZ, Liu DP. Cross-talk between SIRT1 and p66Shc in vascular diseases. *Trends Cardiovasc Med*, 2013, 23: 237–241
  - 25 Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz KT, Gorospe M, de Cabo R, Sinclair DA. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science*, 2004, 305: 390–392
  - 26 Rodier F, Campisi J. Four faces of cellular senescence. *J Cell Biol*, 2011, 192: 547–556
  - 27 Wu WH, Wang SH, Kuan II, Kao YS, Wu PJ, Liang CJ, Chien HF, Kao CH, Huang CJ, Chen YL. Sesamin attenuates intercellular cell adhesion molecule-1 expression *in vitro* in TNF- $\alpha$ -treated human aortic endothelial cells and *in vivo* in apolipoprotein-E-deficient mice. *Mol Nutr Food Res*, 2010, 54: 1340–1350
  - 28 Doller A, Pfeilschifter J, Eberhardt W. Signalling pathways regulating nucleo-cytoplasmic shuttling of the mRNA-binding protein HuR. *Cell Signal*, 2008, 20: 2165–2173
  - 29 Doller A, Schlepckow K, Schwalbe H, Pfeilschifter J, Eberhardt W. Tandem phosphorylation of serines 221 and 318 by protein kinase Cdelta coordinates mRNA binding and nucleocytoplasmic shuttling of HuR. *Mol Cell Biol*, 2010, 30: 1397–1410
  - 30 Amadio M, Bucolo C, Leggio GM, Drago F, Govoni S, Pascale A. The PKC $\beta$ /HuR/VEGF pathway in diabetic retinopathy. *Biochem Pharmacol*, 2010, 80: 1230–1237
  - 31 Kim MY, Hur J, Jeong S. Emerging roles of RNA and RNA-binding protein network in cancer cells. *BMB Rep*, 2009, 42: 125–130
  - 32 Lal A, Mazan-Mamczarz K, Kawai T, Yang X, Martindale JL, Gorospe M. Concurrent versus individual binding of HuR and AUF1 to common labile target mRNAs. *EMBO J*, 2004, 23: 3092–3102
  - 33 Marasa BS, Srikantan S, Martindale JL, Kim MM, Lee EK, Gorospe M, Abdelmohsen K. microRNA profiling in human diploid fibroblasts uncovers miR-519 role in replicative senescence. *Aging (Albany NY)*, 2010, 2: 333–343
  - 34 Abdelmohsen K, Srikantan S, Kuwano Y, Gorospe M. miR-519 reduces cell proliferation by lowering RNA-binding protein HuR levels. *Proc Natl Acad Sci USA*, 2008, 105: 20297–20302
  - 35 Yi J, Chang N, Liu X, Guo G, Xue L, Tong T, Gorospe M, Wang W. Reduced nuclear export of HuR mRNA by HuR is linked to the loss of HuR in replicative senescence. *Nucleic Acids Res*, 2010, 38: 1547–1558
  - 36 Cheng HS, Sivachandran N, Lau A, Boudreau E, Zhao JL, Baltimore D, Delgado-Olguin P, Cybulsky MI, Fish JE. microRNA-146 represses endothelial activation by inhibiting pro-inflammatory pathways. *EMBO Mol Med*, 2013, 5: 949–966
  - 37 Xu F, Zhang X, Lei Y, Liu X, Liu Z, Tong T, Wang W. Loss of repression of HuR translation by miR-16 may be responsible for the elevation of HuR in human breast carcinoma. *J Cell Biochem*, 2010, 111: 727–734
  - 38 Guo X, Wu Y, Hartley RS. microRNA-125a represses cell growth by targeting HuR in breast cancer. *RNA Biol*, 2009, 6: 575–583

**Open Access** This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.